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Study and improvement of spectrophotometric and HPLC methods for the determination of nitazoxanide in pharmaceutical preparations

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ABSTRACT

The different assay methods for the determination of Nitazoxanide in pharmaceutical preparations have been reviewed. The determination of percentage purity of Nitazoxanide has been described by non-aqueous titration method. In this study, Nitazoxanide from commercial pharmaceutical preparations was determined quantitatively employing the modified spectrophotometric and HPLC methods and the methods were compared for their accuracy, specificity and rapidity. The results of both methods were reproducible and within the official limits, however HPLC method has been proved more authentic as it can be used for the determination of percentage purity of raw material of Nitazoxanide and also in combination with other drugs

Keywords: Nitazoxanide , HPLC, Spectrophotometry

INTRODUCTION

Nitazoxanide was originally discovered in the 1980s by Jean-François Rossignol at the Pasteur Institute [1] chemically Nitazoxanide is [2-[(5-nitro-1,3-thiazol-2-yl) carbamoyl] phenyl] ethanoate [2]. Initial studies demonstrated activity versus tapeworms. *In vitro* studies demonstrated much broader activity. Dr. Rossignol co-founded Romark Laboratories, with the goal of bringing nitazoxanide to market as an anti-parasitic drug. Initial studies in the USA were conducted in collaboration with Unimed Pharmaceuticals, Inc. (Marietta, GA) and focused on development of the drug for treatment of cryptosporidiosis in AIDS. Controlled trials began shortly after the advent of effective anti-retroviral therapies. The trials were abandoned due to poor enrollment and the FDA rejected an application based on uncontrolled studies. nitazoxanide was superior to placebo and comparable to metronidazole. Nitazoxanide was successful in the treatment of metronidazole-resistant giardiasis. Studies have suggested efficacy in the treatment of cyclosporiasis, isosporiasis, and amebiasis. [1] It has also been shown to have activity against influenza A virus *in vitro*.[3]

Nitazoxanide is a first-line choice for the treatment of illness caused by *Cryptosporidium parvum* or *Giardia lamblia* infection in immunocompetent adults and children, and is an option to be considered in the treatment of illness caused by other protozoa and/or helminths.[4] It is used for the treatment of infectious diarrhoea caused by *Cryptosporidium parvum*.[4] and *Giardia lamblia*[6] in patients 1 year of age and older. Nitazoxanide is currently in Phase II clinical trials for the treatment of hepatitis C, in combination with peginterferon alfa-2a and ribavirin.[7] [8]

A randomised double-blind placebo-controlled study published in 2006, with a group of 38 young children (Lancet, vol 368, page 124-129[9,10] concluded that a 3-day course of nitazoxanide significantly reduced the duration of rotavirus disease in hospitalized pediatric patients**.** Dose given was "7.5 mg/kg twice daily" and the time of resolution was "31 hours for those given nitazoxanide compared with 75 hours for those in the placebo group." It is

to be noted that rotavirus is the most common infectious agent associated with diNitazoxanide alone has shown preliminary evidence of efficacy in the treatment of chronic hepatitis B over a one year course of therapy.[11] Nitazoxanide 500 mg twice daily resulted in a decrease in serum HBV DNA in all of 4 HBeAg-positive patients, with undetectable HBV DNA in 2 of 4 patients, loss of HBeAg in 3 patients, and loss of HBsAg in one patient. Seven of 8 HBeAg-negative patients treated with nitazoxanide 500 mg twice daily had undetectable HBV DNA and 2 had loss of HBsAg.Additionally, nitazoxanide monotherapy in one case and nitazoxanide plus adefovir in another case resulted in undetectable HBV DNA, loss of HBeAg and loss of HBsAg.[12]

These preliminary studies showed a higher rate of HBsAg loss than any currently licensed therapy for chronic hepatitis B.The similar mechanism of action of interferon and nitazoxanide suggest that stand-alone nitazoxanide therapy or nitazoxanide in concert with nucleos(t)ide analogs have the potential to increase loss of HBsAg, which is the ultimate end-point of therapy. A formal phase Ⅱ study is being planned for 2009.[13] you can see results for off label use of alinia (nitazoxanide) on medhelp hbv community, it is reported a significant drop of more than 50% hbsag quantity from baseline values on hbeag negative hbv at 1,5-2g daily dose (500 mg pills every 6-8hrs with food) already at 3–6 months therapy, especially in combination with peginterferon or entecavir [14].

Romark made the decision to initially focus on the potential treatment of chronic hepatitis C with nitazoxanide. Three phase II studies of nitazoxanide for the treatment of chronic hepatitis C have been completed and communicated in publications or presentations at national and international meetings[6–8]. The first study was a randomized, double-blind, placebo-controlled study of the treatment of chronic hepatitis C with nitazoxanide 500 mg twice daily in 50 adult patients with chronic hepatitis C infected with genotype 4[6]. Seven of 23 patients (30%) had a virologic end-of-treatment response (ETR) with undetectable virus, and a sustained virologic response (SVR) with undetectable virus 24 wk after the completion of treatment was observed in 4 of 23 patients (17%). All responders had low serum HCV RNA levels less than 400 000 IU/mL. This study was the first use of nitazoxanide in patients with chronic hepatitis C and for a longer time period than for its use for cryptosporidiosis and giardiasis, and the drug was well tolerated with the same number of mild gastrointestinal adverse events in the treated and placebo groups [15-18]

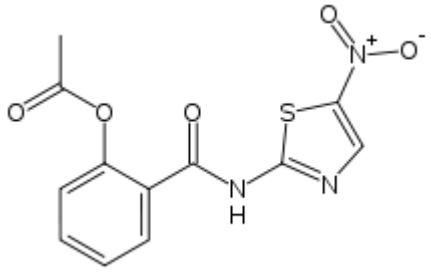
A retrospective review of charts of patients treated with nitazoxanide for trichomoniasis by Michael Dan and Jack D. Sobel demonstrated negative result. They reported three case studies; two of which with metronidazole-resistant infections. In Case 3, they reported the patient to be cured with high divided dose tinidazole therapy. They used a high dosage of the drug (total dose, 14–56 g) than the recommended standard dosage (total dose, 3 g) and observed a significant adverse reaction (poorly tolerated nausea) only with the very high dose (total dose, 56 g). Though the output they got is in favor of safety of the drug but their experience was really disappointing in the treatment of trichomoniasis with nitazoxanide.[19]

In the clinical trials no serious adverse events were reported. No serious adverse events were reported in the prescribing information of the parent company.[20]

Following oral administration, it is rapidly hydrolyzed to its active metabolite, tizoxanide [21]

NTZ has been reported to be active against Ascaris lumbricoides,Cryptosporidium parvum, Cyclospora cayetanensis, Entamoeba histolytica,Encephalitozoon intestinalis, Giardia lamblia, Isospora belli, Trichomonasvaginalis, Vittaforma corneae, Blastocystis hominis, Balantidium coli,Enterocytozoon bieneusi, Trichuris trichura, Taenia saginata, Hymenolepisnana and Fasciola hepatica [22-29]. NTZ is effective in treating common intestinalhelminthes [28-29]. It has also been used for the treatment ofcryptosporidiosis in AIDS patients [30-32].No official method is available in British or US pharmacopoeia for the estimation of NTZ in pharmaceutical ormulation, so far [29]. However, several analytical techniques like liquid chromatography[33-34], adsorptive cathodic stripping voltammetric method [29] Therefore, The aim of the present study was to assess the different methods of quantitative analysis of nitazoxanide and to find out an accurate and rapid method which could be conveniently used for the determination of percentage purity of pharmaceutical nitazoxanide as raw material and also in combination with other drugs. Therefore, in the present investigations various in-house and modified chemical and instrumental methods of analysis including non-aqueous titration, spectrophotometry and HPLC have been applied keeping in view that the recommended analytical method must be sensitive and should give reproducible results

THE CHEMICAL STRUCTURE OF NITAZOXANIDE



Systematic (IUPAC) name of NTZ IS [2-[(5-nitro-1,3-thiazol-2-yl)carbamoyl]phenyl]ethanoate

EXPERIMENTAL SECTION

Materials and chemicals

All the following chemicals were used in this study of analytical grade:Methanol, glacial acetic acid, perchloric acid, acetic anhydride, potassium hydrogen phthalate, crystal violet,NaOH, orthophosphoric acid, and sodium dihydrogen orthophosphate.

Raw material and commercial products

Nitazoxanide raw material was purchased from MANKIND PHARMA ,USV INDIA AND LIOMONT LABS (INDIA) The commercial preparations of Nitazoxanide used in this study were 50 mg tables of each ZINDAX, ZOXAKIND , PARAMIX

Assay of Nitazoxanide raw material by nonaqueous titration

About 250mg of, Nitazoxanide accurately weighed was dissolved in 30 ml of glacial acetic acid, and titrated with 0.1N perchloric acid; the end point was determined by using crystal violet as indicator. The end point was blue to green. Each ml of 0.1N perchloric acid was equivalent to 31.81 mg of Nitazoxanide.

Assay of Nitazoxanide. tablets by spectrophotometric methods

Preparation of standard solution

For spectrophotometeric method 1, the standard sample was prepared as follow Nitazoxanide. standard 50mg was taken and made the volume 100 ml with 0.01N NaOH. Sonicated for 10 minutes, filtered the solution and took 2 ml of filtered solution and made the volume100 ml with 0.01 N NaOH. For the spectrophotometeric method 2, in the above method for standard preparation,0.01N NaOH Was replaced with methanol.

Sample preparation

For spectrophotometeric method 1, the sample was prepared as follow. Twenty tablets of Nitazoxanide were grinded and mixed. The powder sample equivalent to 50 mg of the drug was taken and made the volume to100 ml with 0.01 N, NaOH and sonicated for 10 minutes.The solution was filtered, took 2 ml of filtered solution and made up the volume up to 100 ml with 0.01 N,NaOH. For assay of drug employing spectrophotometeric method 2, same method was used as that employed for the spectrophotometeric method 1 except the Solution 0.01 N, NaOH was replaced with methanol.

Spectrophotmetric assay procedure

All Nitazoxanide commercial preparations were assayed by two spectrophotometric methods, SM1 and SM2. The absorbance of the samples was measured at 330 nm and at 340 nm, for SM1 and SM2, respectively.

Assay of Nitazoxanide tablets by modified B.P

HPLC Methods

Mobile phase for modified HPLC methods

For HPLC method 1, a mixture containing 30 volumes of, equal volumes of a 0.1% w/v solution of orthophosphoric acid and a 0.16% w/v solution of sodium dihydrogen orthophosphate (adjusted to pH 2.5), was prepared with 70 volumes of methanol**.** The mobile phase of the HPLC method 2 consisted of a mixture of 25 volumes of the above solutions, adjusted to pH 2.7,and was mixed with 75 volumes of methanol**.**

Standard solution

Standard solution contained 0.05% w/v and 0.5% w/v of Nitazoxanide in the respective mobile phase for HPLC method 1 and 2, respectively.

Test solution

For HPLC method 1, ten tablets were finely powdered and shaken with 700 ml of methanol (50%) and sonicated for 30 minutes, added sufficient mobile phase to produce 1000 ml. Centrifuged and filtered the supernatant liquid through a 0.45µm filter. The filtrate was diluted with the mobile phase to produce a solution containing 0.05% w/v of Nitazoxanide .For HPLC method 2, the test solution was prepared by using above procedure except the use of 750ml of methanol (50%). The mobile phase for this method was used to produce 0.5% w/v Nitazoxanide solution.

HPLC procedure for drug determination

Separately injected equal volumes (20µl) of the standard solution and the test solution into the chromatograph, recorded the chromatograms, and measured the responses for the main peaks.

RESULTS AND DISCUSSION

In the present study, various chemical and instrumental methods of quantitative analysis have been evaluated for their accuracy, suitability and simplicity in routine industrial analysis. Analysis of Nitazoxanide raw material was performed by non-aqueous titration. Contents of Nitazoxanide in each tablet were between 95-105 %.The percent recovery of the drug in assay using non-aqueous titration method is shown in Table I. A linear relationship between the concentration of raw material and the amount of perchloric acid used proves the validity and accuracy of method. However this method is applicable only for Nitazoxanide raw material and cannot be used for tablets formulations as the excipients may contain some fraction of water especially the film coated tablets.

Table I: Percentage recovery of Nitazoxanide raw material using non-aqueous titration method

Weight of Sample (mg)	Volume of HClO4	% Purity
50	1.57 ml	99.88
100	3.15 ml	100.2
150	4.7 ml	99.67
200	6.3 ml	100.2
250	7.8 ml	99.22

The UV/Visible spectrophotometer method 1 was implemented on Nitazoxanide tablets using 0.01 N NaOH as diluting medium. The results (Table II) were within the official limits and reproducible. Table III shows a linear relationship between the absorbance and concentration of anylate. UV/Visible spectrophotometer method 2 employed methanol as the diluting agent. The findings shown in Table IV were also within the official limits and reproducible. However, the drug showed more absorption in methanol than in NaOH. The result showed a linear relationship between absorbance and concentration of anylate (Table 5). When both in-house methods were compared (Table 6) the results were quite satisfactory.

Table II: Percentage recovery of Nitazoxanide by Spectrophotometric method 1

Sample Tablet	Reference Absorption	Sample Absorption	% Purity
ZINDAX	0.361	0.368	101.9
ZOXAKIND	0.361	0.360	99.72
PARAMIX	0.361	0.356	98.61

Table III: Relationship between concentration and absorbance of Nitazoxanide by spectrophotometric method 1

Weight of Sample (mg)	Concentration (%)	Absorbance
40	**80**	**0.286**
45	**90**	**0.315**
50	**100**	**0.361**
55	**110**	**0.402**
60	**120**	**0.445**

The different brands of Nitazoxanide tablets namely ZINDAX , ZOXAKIND , PARAMIX were analyzed using HPLC. A modified B.P method was used by taking volume of equal mixtures of methanol and phosphate buffer (70:30), and the concentration used was 0.05 w/v. The retention time of Nitazoxanide reduced to 6.5 min as compared to 9.5 min of B.P method where the ratio of mobile phase was (65:35). The results, shown in Table VII were within official limits and reproducible. In HPLC method 2, B.P method with altered composition of the mobile phase (75:25) and the concentration of Nitazoxanide as 0.5 w/v was used. The retention time was reduced to 4.5 min. The method also showed reproducible results as shown in Table VIII. In this study it was also observed that

when the volume of methanol was increased in the mobile phase the retention time for Nitazoxanide was reduced. The findings of the both HPLC methods 1 and 2 were comparable as shown in Table IX. A comparison of the spectrophotometric and the modified HPLC B.P methods (Table X) did not show any significant variation and proved the accuracy, validity and specificity of these methods

Table IV: Percentage recovery of Nitazoxanide by spectrophotometric method 2

Sample Tablet	Reference Absorption	Sample Absorption	% Purity
ZINDAX	0.453	0.459	101.32
ZOXAKIND	0.453	0.470	103.75
PARAMIX	0.453	0.471	103.97

Table V: Relationship between concentration and absorbance of Nitazoxanide by spectrophotometricmethod 2

Weight of Sample (mg)	Concentration (%)	Absorbance
40	80	0.360
45	90	0.430
50	100	0..448
55	110	0.490
60	120	0.531

Table VI: Comparison of % purity of both spectrophotometric methods

Sample methods	% Purity by spectrophotometric methods	
	Method 1	Method 2
ZINDAX	**101.9**	**101.32**
ZOXAKIND	**99.42**	**103.75**
PARAMIX	**98.61**	**103.97**

Table VII: Percentage recovery of Nitazoxanide by B.P., HPLC modified method 1

Sample Tablet	Reference Area	Sample Area	% Purity
ZINDAX	2767499	2782055	100.53
ZOXAKIND	2767499	2741357	99.05
PARAMIX	2767499	2812921	101.64

Table VIII: Percentage recovery of Nitazoxanide by B.P., HPLC method 2

Sample Tablet	Reference Area	Sample Area	% Purity
ZINDAX	15490	15635	100.93
ZOXAKIND	15490	15863	102.40
PARAMIX	15490	15616	100.81

Table IX: Comparison of % recovery of both B.P., HPLC methods, 1 and 2.

Sample Tablet	% purity Sample Tablet by HPLC methods	
	Method No 1	Method No 2
ZINDAX	100.53%	100.93%
ZOXAKIND	99.05%	102.40%
PARAMIX	101.64%	100.81%

Table X: Comparison of results of spectrophotometric and B.P., HPLC methods

Sample Tablet	Spectrophotometer Method (% purity)		HPLC methods (% purity)	
	Method No 1	Method No 2	Method No 1	Method No 2
ZINDAX	101.9	101.32	100.53%	100.93%
ZOXAKIND	99.42	103.75	99.05%	102.40%
PARAMIX	98.61	103.97	101.64%	100.81%

The present findings suggest that by using either method,the percentage purity of the raw material and the commercial products could be determined, accurately and reproducibly. The excipients in the formulation did not interfere with the findings with HPLC. These finding indicated that the above method are efficient and reliable analytical technique for routine quality control analysis of raw material and drugs.

CONCLUSION

The developed method was found to be simple, sensitive, accurate, precise, economic and can be used for routine quality control analysis of Nitazoxanide in bulk as well as in pharmaceutical dosage form.

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